

eIF3 is a multi-subunit translation factor which promotes translation in favor of carcinogenesis. It binds mRNA to the ribosome, but the specific mRNA features responsible for eIF3-dependent translation remain to be determined. The g subunit of eIF3 (eIF3g) carries the RNA-binding motif. eIF3g binding to the main body of eIF3 is mediated by its interaction with the i subunit of eIF3. By genome-wide translation profiling studies of an eIF3i mutant, we found that eIF3i promotes translation of mRNAs whose 5' UTR is enriched with certain mRNA motifs including the ACAA motif. In his project, Eric will construct luciferase reporter plasmids whose start codon is preceded by these motifs and examine if they promote eIF3i-dependent translation using luciferase assays, and if so determine which nucleotide of the motifs is important by mutational approaches. During the semester, Eric modified his experimental approach to utilize a technique more like spot assay procedure than that which used a lysate. These modified experiments were shown to have more consistent results and it is suspected that the variance seen within the original technique was due to a single copy plasmid copy number variation. Overall, it was found that motifs 1-3 promote translation and that this effect was amplified in a tif34 background. It is still difficult to tell why there are differences between the three motifs, but motif1 consistently gives the highest values. Motif1 very clearly promotes translation and plays a more important role in the absence of tif34 than in its presence. In the future, additional experiments will be performed to determine exactly which nucleotides play a role in effecting translation.